

Effect of phospholipase C on calcium release from epithelia treated with antidiuretic hormone

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Summary

1. Addition of antidiuretic hormone (ADH) to the bladders of toads (*Bufo marinus*) preloaded with ^{45}Ca causes an increase in the rate of calcium efflux.
2. Pretreatment of the serosal surfaces of bladders with phospholipase C prevents the action of ADH on calcium efflux.

Introduction

When toad bladders are treated on the serosal side with antidiuretic hormone (ADH) the permeability of the tissue to water increases. This can be measured easily as a mucosal to serosal water movement when bladders are subjected to an osmotic gradient with a hypotonic solution bathing the mucosal surface (Bentley, 1958). Some time ago (Cuthbert, Ind & Wong, 1971) we reported that when the serosal surfaces of bladders are treated with phospholipase C the hydro-osmotic effect of ADH is abolished. However the most interesting point concerned with this finding was that enzyme treatment had no effect on the generation of cyclic adenosine monophosphate (cAMP) in bladders stimulated with ADH. In this tissue, as well as others sensitive to ADH, cAMP is believed to be the second messenger which is responsible for the permeability changes (Handler & Orloff, 1971). It is known that the permeability change to water occurs at the mucosal face (Hays & Leaf, 1962) even though the hormone acts only at the serosal side.

In this paper we have investigated the possibility that ADH might trigger other events, as well as cAMP generation, which are blocked by pretreatment of the serosal surface by phospholipase C. It is known that when bladder tissues are preloaded with ^{45}Ca there is an increased efflux of label in the presence of ADH (Schwartz & Walter, 1968). Here we show that phospholipase C applied to the serosal surface abolishes the effect of ADH on calcium efflux.

Methods

Bladders were removed from toads (*Bufo marinus*) and the two lobes filled with Ringer solution and tied to form balloons with the serosal surfaces outermost. One bladder lobe from each toad was placed in oxygenated Ringer solution while the other was placed in a similar solution containing phospholipase C (100 $\mu\text{g}/\text{ml}$, Type I from *Clostridium welchii*, 4 u/mg, Sigma). Both control and test bladders were incubated for 1 hour at room temperature. Subsequently the bladders were washed in Ringer solution and 1 cm square pieces cut from each lobe. The bladder pieces were placed in oxygenated Ringer solution containing ^{45}Ca (10 μCi in 20 ml) for 1 hour. Fine threads were then attached to the pieces which were lowered

in turn into a series of 10 tubes containing 2 ml Ringer solution, the tissue remaining in each tube for 10 minutes. The tubes were gently bubbled with air to promote mixing. Tubes 6–10 contained ADH (Pitressin, Parke Davis, 50 mu/ml). The ^{45}Ca released during 10 min was measured by taking 1 ml from each tube and counting by liquid scintillation spectrometry. This experiment was repeated five times.

The Ringer solution used throughout was as follows (mM) NaCl, 112; KCl, 3.5; CaCl_2 , 1.0; NaH_2PO_4 , 0.08; NaHCO_3 , 2.4; and glucose 11.1. This solution had a pH of 7.6 when equilibrated with air.

Results

Figure 1a shows the results from five experiments in which the release of ^{45}Ca from control and phospholipase C-treated bladder pieces was compared. It can be seen that ADH causes an increased calcium efflux only in those tissues which were not treated with phospholipase C. Semilogarithmic plots of the same data are shown in Figures 1b and c. The efflux of ^{45}Ca from the treated tissues has a double exponential form with rate constants of 0.12 min^{-1} and 0.017 min^{-1} . Assuming that the efflux from the control bladders would have had the same rate characteristics if ADH had not been added, an assumption which seems justifiable from the early part of the curves, then the total amount of ^{45}Ca present in both treated and untreated tissues can be calculated. The efflux of ^{45}Ca from the tissues can be expressed as the washout rate constant, f , which is the fractional rate of loss in unit time. This is given by $f = \frac{\Delta C}{\Delta t \text{ Ct}}$

where ΔC represents the counts lost per interval (10 minutes), Δt represents the interval and Ct is the total number of counts remaining in the tissue half-way

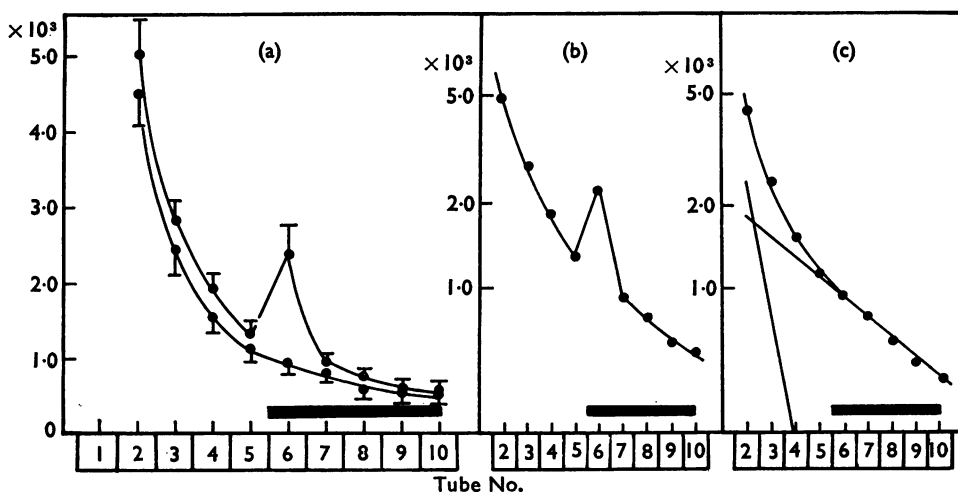


FIG. 1. The release of ^{45}Ca from toad bladder pieces. The activity released in counts/min per ml of bathing solution is shown on the ordinate. Note that the scales in (b) and (c) are logarithmic. The abscissae show the tube number, each interval being equivalent to a 10 minute efflux period. In (a) the upper curve relates to control tissues and the lower curve to phospholipase C-treated tissues. These two curves are replotted on a semilogarithmic scale in (b) and (c) respectively. The results in (a) show the means and standard errors for 5 experiments. Antidiuretic hormone (50 mu/ml) was present in tubes 6–10 (shown by horizontal bar). In (c) the two exponents shown were derived by curve stripping.

through the interval. Ct is obtained from the difference between the total number of counts present initially minus those lost up to that period.

It can be seen from Figure 2 that in control tissues ADH causes an increase in f for a period of at least 40 min, that is for as long as efflux was measured in the presence of hormone. In contrast tissues treated on the serosal side with phospholipase C failed to show any increase in efflux in the presence of hormone.

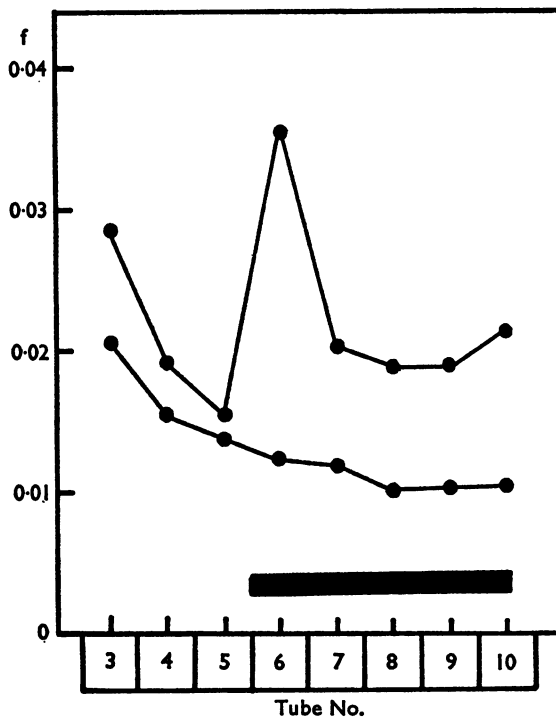


FIG. 2. Effect of antidiuretic hormone (ADH) on efflux of ^{45}Ca from toad bladder pieces expressed as the washout rate constant, f . ADH (50 mu/ml) was present as indicated. The upper curve relates to control tissues and the lower curve to phospholipase C-treated tissues.

Discussion

This report, taken together with our previous findings (Cuthbert *et al.*, 1971) shows that ADH causes not only an increase in tissue cAMP levels but an increase in calcium efflux. Furthermore treatment of the serosal surface of bladders with phospholipase C blocks the increase in calcium efflux and the hydro-osmotic response to ADH, while the generation of cAMP in the tissue remains unaffected. Thus the inhibition of the physiological response to ADH by enzyme treatment correlates with the inhibition of calcium efflux.

From other studies on toad bladder it has been suggested that calcium release from the mucosal membrane might be the final effector process by which the permeability of the mucosal face of the epithelium is changed (Cuthbert & Wong, 1971). Although phospholipase C treatment inhibits both calcium release and the hydro-osmotic response it cannot be assumed that the calcium is released from the outer mucosal face. In other (unpublished) experiments we have shown that ADH causes increased efflux only from the mucosal and not the serosal surface

of the bladder. Thus the calcium might arise within the cells or from the mucosal surface. This finding, however, eliminates the possibility that the increased efflux may have come from muscle fibres on the serosal side. However, treatment of the mucosal face of bladders with ethylene glycol bis-(2-aminoethyl tetra-acetic acid) to remove bound calcium only produces a minor increase in water permeability compared to ADH (Wong, Bedwani, & Cuthbert, 1972).

The present findings can be considered in three ways. First phospholipase C may cause changes which interfere with the action of cAMP after its generation and which result in the failure of calcium release. For example, cAMP might activate a protein kinase which alters the calcium binding properties of cell membranes. In this context it must be remembered that phospholipase is used to treat only the serosal side but that the permeability change occurs at the mucosal surface (Hays & Leaf, 1962). Alternatively ADH may cause other changes at the serosal faces of the cells, as well as stimulation of adenyl cyclase, which are necessary for calcium release and the expression of the physiological response, and which are prevented by phospholipase pretreatment. A further possibility is that stimulation of adenyl cyclase together with other events are necessary to generate the permeability change. Further speculation is not profitable until the precise source of the calcium released by the hormone is known.

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